Genetic diversity and effect of selection at the mitochondrial hypervariable region in major Nigerian indigenous goat breeds

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Abstract

Mitochondrial DNA (mtDNA) hypervariable region 1 (HV1) sequences of three Nigerian indigenous goat breeds; West African Dwarf (WAD), Red Sokoto (RS) and Sahel were used to investigate the genetic diversity and effect of selection between and among these populations. Deoxyribonucleic acid (DNA) of Nigerian indigenous chicken was extracted from blood samples collected and preserved on Fast Technology for Analysis (FTA) paper. The extracted DNA were amplified and sequenced with predefined mitochondria (mtDNA) primer sets for HV1. Eighty-seven (87) polymorphic sites were found in 115 sequences which were grouped into 92 haplotypes. The mean haplotypic and nucleotide diversity were found to be 0.996 ± 0.002 and 0.092 ± 0.04 respectively. Genetic variation within population and between populations accounted for 97.26% and 2.74% of the total maternal variation respectively, with F_{ST} value of 0.0274. The Tajima's (D) and Fu's (F) test of neutrality were significant ($P<0.05$) and negative with the mean value of -1.12 and -21.34 respectively which is an indication of population expansion. The result further revealed that the WAD and RS goats are closely related with less genetic distance value of 0.01, and high genetic distance value (0.02) was observed between RS and Sahel goats and WAD and Sahel goats. Selection analysis result shows that there is more positive selection site (6 sites) to negative

site (5 sites) among the Nigerian goats, which signifies how diverse they are as well as how nature has being trying to confer genetic fitness to these breeds.

Keywords: *Goat breeds; Mitochondrial DNA; Genetic diversity; Selection*

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Introduction

Domestic goats (*Capra hircus*) were among the first domesticated animals adapted from the wild *Capra aegargus* at about 10,000 years ago (Sardina *et al*., 2006; Naderi *et al*., 2007) and studies have revealed that they were introduced into the African continent from Southwest Asia (Pereira *et al*., 2009). Goat is one of the most important, adaptable and geographically widespread livestock species (Carl and Kees, 2004), which provides a good source of meat, milk and other by-products and are therefore referred to as "poor man's cow" (MacHugh and Bradley, 2001).

There are three recognized indigenous breeds of goats in Nigeria: the hardy short-legged West African Dwarf which is confined to the humid forest belt, the relatively small sized Red Sokoto found in the semi-desert and the long-legged Sahel found in the Savannah zone of the country (Adu and Brinckman, 1980). The Red Sokoto (a meat type animal) is characterized by its uniformly dark red coat colour, short and horizontal ear, and horns in both sexes (Adu and Brinckman, 1980) and among the most valuable of goat skins which commands a premium in the world market. The West African Dwarf (WAD) goats (a meat and milk type animal) resemble Red Sokoto goats in body proportions. The Sahel goat (meat, milk and skin type animal) has varying coat colours usually white pied with black or brown, long legs, and long and horizontal, but sometimes moderately long and pendulous ear. The horns are long, flat and twisted in the male, but sickle shaped in female (Devendra and Burns, 1983).

Genetic diversity of animals is that characteristics that enable different animals to survive in different climatic zones, for example, the WAD is able to thrive in trypano-endemic region of the humid tropical region while the Sahel goat thrives well in the Savannah zone of Nigeria due to their genetic diversity. Domestic animal diversity is important for food security, to meet the demand due to population expansion, climate change and disease outbreak. While some studies have been carried out on morphometric characterization of Nigerian goat (Okpeku *et al*., 2011; Yakubu *et al*., 2010), little or no work has been done on the molecular characterization existing within and between this species in Nigeria. The study of the polymorphisms in mitochondria DNA in the various populations would help to clarify the ancestral lineage and to compare the populations. For effective utilization of goat genetic resources, it is necessary to genetically characterize different populations. Such characterization would provide a database with information on which of the populations represent homogeneous breeds and which are genetically distinct. This characterization tends to contributes meaningful to the understanding of the evolutionary history as well as future conservation and management of goat genetic resources.

The detection and quantification of evolutionary (selection) pressures that have contributed to genetic variation has been an active area of recent research, and several statistical methods (Nielsen and Yang, 1998; Suzuki and Gojobori, 1999; Yang *et al*., 2000; Nielsen and Huelsenbeck, 2002; Suzuki, 2004; Huelsenbeck and Dyer, 2004) have been described for the identification of newly arisen mutation which has a selective advantage over others, leading to either positive (diversifying), negative (purifying) or balancing (neutral) selection. Majority of these methods rely upon estimating site-specific synonymous (d_S) and non-synonymous (d_N) substitution rate parameters, and performing statistical tests to determine whether $d_S \neq d_N$ (Yang, 2002; Kosakovsky Pond *et al*., 2005). Identification of selection pressure surrounding candidate gene or loci in any population will help to identify important genes necessary for production or adaptation. The analysis helps to identify regions or loci that are significantly different between breeds and therefore may be genetic variants targeted by selective breeding. This present study

aimed to analyze genetic diversity and effect of selection in the three major Nigerian goat breeds (Red Sokoto, Sahel and West African Dwarf).

Materials and Methods

Sampling and DNA Isolation

A total of 115 goat blood samples were collected from three Nigerian indigenous goats (Sahel (S), n = 45, West African Dwarf (WAD), n = 34 and Red Sokoto (RS), n= 36) from three geographical zones of Nigeria. The blood were collected by vena puncture and transferred immediately to FTA® classic cards (Whatman BioScience, Maidstone, UK) to dry. To ensure that the goat populations that were sampled were different and to prevent sampling crossbred animals, sampling areas were at least 50 km apart. From the FTA® classic cards, 1 mm disk of the blood sample was punched and put in a 1.5 mL Eppendorf tube for each sample collected. Later, 50 µL ddH_20 was added and vortexed three times and left to rest for 10 min. The spent water was removed as much as possible. Then, 100 µL of ddH_2O was added so as to submerge the disks. The tube with the disks were then transferred to a heating block and heated at 99 ℃ for 15 min. The samples were vortexed and briefly centrifuged. The extract was pipetted and was put in a new tube; the preparation was having 60 – 150 µL of DNA of between 40 – 80 ng of DNA.

PCR amplification

Using the primers: CAP - F (5′ - CGTGTATGCAAGTACATTAC - 3′) and CAP - R (5′ - CTGATTAGTCATTAGTCCATC - 3′), polymerase chain reaction was carried out using 30 µl reaction volume containing 2.5 mM of each dNTPs, 14 pmol of each primer, 1.5 mM $MgCl_2$, 1 × PCR buffer comprising 10 mM Tris-HCl (pH 8.3) and 50 mM KCl, and 1.25 U Taq DNA polymerase (Roche Applied Sciences, Germany). Amplifications were carried using the GeneAmp® PCR system 9700 thermal cycler (Applied Biosystems, Foster City, USA) programmed as follows: initial denaturation of 94 ^oC for 2 min, followed by 10 cycles at 94 ^oC for 15 sec, 58 ^oC for 30 sec and 72 ^oC for 40 sec.

Sequencing of the mtDNA

The sequencing was carried out using 20 µl comprising approximate 20 ng of purified PCR product as template DNA, 3.2 pmol of primer and 8 µl of Big Dye Terminator Ready Reaction Mix (mixture of dNTPs, ddNTPs, buffer, enzyme and $MgCl_2$), 8 µl of deionized water, 2 µl of primer and 2 µl template DNA, using a ABI 3730 XL Capillary DNA Analyzer (Applied Biosystems, Foster City, USA) programmed as: 25 cycles at 96 ^{o}C for 10 seconds, 50 ^{o}C for 5 seconds and 60 ^{o}C for 4 minutes. After the last cycle, there was a rapid thermal ramp to 4 ^{o}C and holding until the purification of the sequencing product.

Data Analysis

A 453bp long fragment, including the hypervariable region 1 (HV1), was subsequently used for analysis. mtDNA polymorphisms were identified in a total of 115 sequences of 3 breeds analyzed and aligned with BioEdit software (Hall, 1999). Multiple alignments of the sequences excluding all the gap was performed using ClustalW in MEGA 6.06. DnaSP software (Librado and Rozas, 2009) was used to calculate haplotype, sequence variation, average number of nucleotide difference and average number of nucleotide substitutions per site between breeds. A Neighbour joining tree (NJ) was constructed with MEGA 6.06 software (Tamura *et al.*, 2013) using the identified haplotype among the goat breeds. A Median Joining (MJ) network analysis was constructed with the observed haplotypes and wild goat sequences using NETWORK 4.6.1.2 software (Bandelt *et al*. 1999). Genetic differentiation based on the analysis of molecular variance (AMOVA), mismatch distributions, Tajima's *D* and Fu's *Fs* were calculated using Arlequin 3.5.1.3 software (Excoffier *et al*., 2005). The generated mtDNA sequences were used in testing for among and within selection effect based on Single likelihood ancestral counting (SLAC) method using Hyphy 2.2 software (Kosakovsky Pond *et al.*, 2005; available at http: //www.hyphy.org.)

Results

Mitochondrial diversity indices

The DNA segment sequenced was 453bp long fragment, including the hypervariable region 1 (HV1), from PCR amplified products and have been subsequently deposited into the GenBank (GenBank accession number KU292651-KU292742). The result of genetic diversity indices were shown in Table 1. High haplotypic (gene) diversity and nucleotide diversity were found among the populations. In consistence with the fixation index (F_{ST}) results, Red Sokoto and Sahel goats had the highest haplotypic (gene) diversity of 0.99±0.08 and 0.99±0.01 respectively while the least value (0.98±0.01) was observed in WAD goats, with a mean value of 0.996±0.002 for the sampled populations. The highest nucleotide diversity value was observed in Sahel goats (0.11±0.06) and the least value (0.08±0.04) was observed in Red Sokoto and WAD goats, with a mean value of 0.092±0.04 for the sampled populations. Sahel goat had the highest mean number of pairwise difference (9.32±4.36) while WAD goat had the least value of 6.55±3.17, and a mean value of 7.66±1.46. Table 2 shows AMOVA result which reveals higher maternal genetic variation (97.26%) within population and 2.74% between populations, with a fixation index (F_{ST}) value of 0.0274

Mismatch distribution

The result of Fu's *F*s and Tajima's *D* tests of neutrality carried out to detect population expansions under sudden expansion model were shown in Table 3. The overall validity of the estimated demographic model was tested by obtaining the goodness-of-fit of the observed data to the simulated model of expansion with *SSD* and *r* obtained by a bootstrap approach (Schneider and Excoffier, 1999). The *P* values of the statistics were computed as the proportion of simulated cases that show values larger than the original. A significant *r* or *SSD* value was taken here as evidence for departure from the estimated demographic model, which can be either a model of population expansion (if $t > 0$ and $\theta_1 > \theta_0$) or a model of population stationary (if $t = 0$ or $\theta_1 > \theta_0$).

The mismatch distribution analysis shows genetic signature of population expansion within and between the three goat breeds (Figure 1 – 3). The mismatch distribution had a multimodal curve with three (3) major peaks at 7, 9 and 11 differences in Red Sokoto population, three major peaks at 3, 12, 15 differences and 2 minor peak at differences 24 and 29 in Sahel goat population, and two major peaks at around 2 and 7 differences in WAD. Sahel breed has the highest unweighted mean pairwise difference of 9.317, followed by Red Sokoto (6.735) with WAD having the least of 6.545.

Phylogenetic and Network analyses

The result of the Neighbor joining analysis based on Tajima and Nei (1984) model using bootstrap method (1000 replications) revealed that identified sampled Nigerian goat haplotypes clustered around *Capra aegagrus* with a sum of branch length (SBL) of 0.79 as shown in Figure 4. The Median joining network was drawn for the haplotypes identified in 115 Nigeria goat samples and 6 wild goat haplotypes. The network illustrates the relationship between haplotypes in sampled Nigerian goat breeds and wild goat haplotypes. The observed 92 Nigerian goat haplotypes clustered around *Capra aegagrus*, and are far apart from other *Capra* breeds as shown in Figure 5.

Genetic distance

The genetic distance estimated between populations was found to be 0.0191 between WAD and Sahel, 0.0194 between Red Sokoto and Sahel, and 0.0156 between WAD and Red Sokoto (Table 4). The distance within populations was observed to be 0.0162, 0.0214 and 0.0149 for Red Sokoto, Sahel and West African Dwarf goat respectively.

Selection detection between and within the three Nigerian goat breeds

The result of selection analysis ($p < 0.05$) carried out to detect the selection within and between three Nigerian goat breed are shown in Table 5. Red Sokoto breed has one and equal number of positively and negatively selected site, but, at the different site index, 1 positively and 2 negatively selected sites were found in Sahel, while WAD has only 1 negatively selected site with no positively selected site. Six positively and 5 negatively selected site were found between the three Nigerian goat breed when the sequences of the three breeds were pooled together (Table 5b)

Discussion

Studies involving the use of mitochondrial DNA sequences have shown that goat breeds have high nucleotide and haplotype diversities indicating high genetic variations among different goat populations (Naderi *et al*., 2008). Goat also has weak large-scale population structuring than other livestock species suggesting that there has been high rate of interbreeding and exchange of goat genetic resource (Fernandez *et al.*, 2006) in the course of domestication and selection. The aim of this study was to have an understanding of the genetic diversity and level of admixture in Nigerian goats. Nucleotide diversity and haplotype diversity have been described as the important indices for assessing population polymorphisms and genetic differentiations, that is, the higher the two indices of population mtDNA, the higher the population polymorphisms (Liu *et al*., 2007).

The mitochondrial HV1 region was highly variable among Red Sokoto, Sahel and WAD goats as revealed by high haplotype and nucleotide diversities. The estimates observed were comparable with haplotype and nucleotide diversity estimates reported in previous similar studies (Sardina *et al*., 2006; Zhao *et al*., 2011). This high variability reflected the diversity already present in the first domesticated individuals that arrived in Nigeria when compared with many world goats (Naderi *et al*., 2007). The level of diversity is not unexpected as this region is not known to code for any functional protein and has been identified as a mutational hotspot (Stoneking, 2000). This result revealed that Red Sokoto and Sahel goats have high

genetic diversity among the three Nigerian breeds. The lowest genetic diversity observed in WAD could be attributed to the fact that 90% of WAD goats in Nigeria are owned by smallholder rural goat keepers which are generally kept in small herds on mixed farms or due to other factors like genetic drift or bottleneck. One of reasons for this low genetic distance may be attributed to transportation for commercial trade (North – South movement) or during migratory and exploratory movements of humans (Luikart *et al*., 2001).

Geographic structuring was weak among the three goat breeds which is in accordance with previous studies carried out on domestic goats by Naderi *et al*. (2007), and reports from several regions also shows weak or lack of geographical structure (Zhao *et al*., 2011). The weak geographic structure in domestic goat has been explained by a high transportation of this species in relation to human migration and commercial trade (Fernandez *et al*., 2006). Likewise, the use of fewer vigorous bucks to mate numerous does in order to produce viable kids to increase profitability by the rearers coupled with indiscriminate mating among these animals as they are mostly kept under extensive management system of production reduce heterogeneity within this population.

Tajima's *D*, Fu's *F* and mismatch analysis were used to examine the traces of population expansion. Results of both statistics (Tajima's *D* and Fu's *F*) were significant ($p < 0.05$) are negative, suggesting the existence of population expansion. Test of validity was non-significant implying similarity in simulated and observed distributions inconsistent with Tajima's D and Fu's *F*. The test statistics also show positive values, indicating a deficiency of low-frequency mutations. A significant negative *F* value indicates excess of rare mutations which is a pattern commonly attributed to a normal growing population. This was observed in the three Nigerian goat breeds. The multimodal curve of the mismatch analysis revealed a recent population expansion as revealed by the major peaks within each breeds of Nigerian goats. The 3 and 2 major peaks within Red Sokoto and WAD goats respectively suggests that at least three and two expansion events occur among the goat population respectively, while three (3) major and two (2) smaller

peaks were observed in Sahel goat population. The highest genetic distance was observed between Sahel and the other two goat breeds while Red Sokoto and WAD goats has the lowest genetic distance which may be due to geographic connection between RS and WAD and which might have allowed some level of gene flow between them. On the other hand, there is high geographic distance between the Sahel breed and the other two breeds which might have influence the level of genetic distance observed in this study.

The control region of mtDNA has the main regulatory elements for mtDNA replication and transcription. Mitochondrial also function for energy metabolism, with 36 molecules of adenosine triphosphate (ATP) per glucose molecule in contrast to the 2 ATP molecules produced by glycolysis, production of reactive oxygen species and apoptotic pathways. Mitochondria function has been implicated in physical performance and influence of functional decline and vulnerability to diseases in later life. It has been reported that polymorphisms in the control region of human mtDNA is associated with elite Japanese athlete status and may influence their physical performance (Mikami *et al*., 2012). It also influences key indices of trainability in aerobic capacity, for example, VO_{2max}, citrate synthase activity and mtDNA content in skeletal muscle (Marakami *et al*., 2002). Significant negative values of neutrality tests can be an indication of selection but are also consistent with either population subdivision or expansion Ray *et al*., 2003). To determine whether purifying or adaptive selection influenced the evolution of Nigerian goat mtDNA, we compared the number of nonsynonymous to synonymous substitutions, and the ratios of nonsynonymous substitutions per nonsynonymous site to synonymous substitutions per synonymous sites (d_N/d_S) in each goat population and the pooled samples. The high rate of synonymous substitutions in WAD where d_N/d_S is low and lack of positively site, indicate that purifying selection has been in influential in WAD mtDNA evolution (normalizing selection i.e. preserves formed adaptations). This process of purifying selection is a form of natural selection responsible for the preservation of adaptive characteristics under constant environmental conditions. Charlesworth (2006) opined that purifying selection reduces the frequency of deleterious allele and the genetic diversity at linked loci as well as effective population size, genetic variability and gene frequency. Likewise, in Sahel population, there is

occurrence of purifying selection where only one site is positively selected while two are under the influence of negative or purifying selection. On the other hand, the Red Sokoto breed is having equal number of positive and negatively sites which is compatible with neutral evolution. Purifying selection or excess of nonsynonymous substitutions in mtDNA coding regions have been reported in wolves, coyotes, dogs, mice and human (Björnerfeldt *et al*., 2006; Sun et al., 2006; Howell *et al*., 2007; Stewart *et al*., 2008). The difference in d_N/d_S ratios between these populations is not unusual given that mutations can be neutral in some lineages but not in others (Moilanen *et al*., 2003). Polymorphisms in mtDNA control regions have been implicated in the level of physical performance or fitness in human (Marakami *et al*., 2002; Mikami *et al*., 2012). The positively selected sites in Red Sokoto and Sahel breeds might confer selective advantage for certain haplotypes in hot climates and influence their adaptation to the hot climate region of the northern Nigeria where these populations are adapted. On the other hand, the WAD is found mainly in the humid tropical zones of southern Nigeria endemic with trypanosomiasis. The negatively selected site observed WAD goat population may due to genetic drift, human migration and exchange through trade, high incidence of commercial activities as well as random mating pattern, which could have resulted in heterozygous deficiency and insufficient time to establish a balance between the occurrence of new mutations and their genetic diversity loss. The Red Sokoto and Sahel breeds are adapted to the dry northern Nigeria characterized by undernutrition during the extended dry season and had to trek longer distances in search of forages. Therefore, the positively selected sites may have selective advantage in term of physical fitness to these breeds when compared to WAD goats which no positively selected site.

In general, the pooled sample of Nigerian goat breeds show more positive than negatively selected sites in their mtDNA control region indicating some alleles with selective advantage for genetic fitness and adaptation to the geographical agroclimatic zones of Nigeria and shows that some level of genetic diversity still exist within the Nigerian goats. Although mitochondria evolution is more complex, our data suggests that climate adaptation may influence the evolution of mtDNA lineages in Nigerian goats.

Further investigation with larger dataset with an alternative approach is suggested and could provide valuable insight into the role of climate in shaping the distribution of goat mtDNA in Nigeria and to separate demographic histories from signal of adaptive evolution.

Conclusion

Selection has greatly contributed to the high genetic diversity of Nigerian goats, with Red Sokoto breed being neutral to the selective pressure. A broad and well-defined breeding and conservation programme including the government, researcher and livestock farmers needs to be put in place in order to address the effect of selection among Nigerian indigenous goat populations. The lack of genetic substructure in the goat populations is evidence of a single maternal origin and extensive genetic intermixing in the past and present times.

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Table 1: Genetic diversity indices of Nigerian indigenous goat breeds

Diversity Indices	Red Sokoto	Sahel	WAD	Mean
Sample size	36	45	34	38.33±5.86
Haplotypes	31	37	27	31.67±5.03
Gene diversity (Hd)	0.99±0.01	0.99±0.01	0.98±0.01	0.996±0.002
Sum of square freq.	0.04	0.03	0.05	0.013
Number of observed transitions	42	63	35	46.67±14.57
Number of observed transversions	1	7	1	3.00±3.46
Number of substitutions	43	70	36	49.67±17.95
Number of polymorphic sites	43	67	36	48.67±16.26
Nucleotide diversity (±) Standard deviation	0.08±0.04	0.11±0.06	0.08±0.04	0.092±0.04
Mean Number of pairwise difference	7.12±3.42	9.32±4.36	6.55±3.17	7.66±1.46
θ_s	10.37±3.40	15.32±4.65	8.81±2.97	11.50±3.67
θ_π	7.12±3.80	9.32±4.84	6.55±3.53	7.66±4.06
C (%)	20.31	20.15	20.18	20.21
T (%)	33.68	33.97	33.77	33.82
A (%)	22.35	22.40	23.09	22.59
G (%)	23.66	23.47	22.95	23.38

θ_s: Theta value based on number of segregating sites; θ_π: Theta value based on the average number of pairwise differences

Table 2: AMOVA showing genetic variation between and within Nigerian goat breeds

SOV	d.f	SS	Variance components	% variation	F_{ST}
Among pops	2	16.181	0.11002 Va	2.74	0.0274
Within pops	112	437.506	3.90630 Vb	97.26	
Total	114	453.687	4.01631		

SOV=Source of variation; d.f. =degree of freedom; SS=Sum of squares; F_{ST} = fixation index

Table 3: Demographic expansion indices of Nigerian goat breeds

Indices	**Red Sokoto**	**Sahel**	**WAD**	**Mean**
Mismatch observed mean	7.12	9.32	6.55	7.96
Mismatch observed variance	9.42	18.54	7.56	13.24
T	5.57	9.63	5.31	6.84
θ_0	0.00	0.044	1.36	0.47
θ_1	306.88	56.93	99999.0	33454.27
Tajima's D	-1.13	-1.39	-0.92	-1.15
P (D simul < D obs)	0.14	0.06	0.20	0.13
Fu's F	-23.28	-23.57	-17.18	-21.34
P (sim_Fs < = obs_Fs)	0.00	0.00	0.00	0.00
Harpending's Raggedness index	0.010	0.010	0.010	0.010
P *(Sim. Rag. > = Obs. Rag.)*	0.9	0.83	0.68	0.80
Sum of square deviation (*SSD*)	0.024	0.001	0.001	0.010
P *(Sim. SSD > = Obs. SSD)*	0.00	0.90	0.71	0.54

T = time of expansion; θ_0 and θ_1: mutation parameters

Table 4: Genetic distance within and between Nigerian goat breeds

Breed	Red Sokoto	Sahel	WAD
Red Sokoto	****	0.0194	0.0156
Sahel	0.0162	****	0.0191
WAD	0.0149	0.0214	****

Genetic distance within (below diagonal) and between (above diagonal) Nigerian goat breeds. WAD=West African Dwarf

Table 5a: Selection analysis result for within Nigerian goat breeds

Breed	Selection type	Site index	Observed NS changes	d_N	d_S	$d_N - d_S$ value	*p*-value
Red Sokoto	Positive	5	4.000	9.284	2.343	6.941	0.010
	Negative	11	7.000	0.998	7.025	-6.027	0.008
Sahel	Positive	19	3.000	12.156	1.698	10.457	0.000
	Negative	9	14.000	1.487	14.543	-13.065	0.000
		13	16.000	1.983	16.274	-14.290	0.000
WAD	Positive	-	-	-	-	-	-
	Negative	9	9.000	0.996	9.066	-8.070	0.001

WAD: West African Dwarf

Table 5b: Selection analysis result for between Nigerian goat breeds

Breed	Selection type	Site index	Observed NS changes	d*N*	d*S*	d*N* - d*S* value	*p*-value
Nigerian goat breeds	Positive	7	1.000	9.998	1.000	8.997	0.002
		8	2.000	8.457	2.058	6.399	0.028
		10	10.500	23.969	8.860	15.109	0.002
		14	3.000	11.997	3.002	8.995	0.008
		16	3.500	23.745	3.235	20.510	0.000
		17	1.000	6.957	1.013	5.944	0.021
	Negative	9	6.000	1.500	6.000	-4.500	0.042
		12	23.000	1.100	23.004	-21.004	0.000
		15	13.000	2.500	13.002	-10.502	0.001
		20	12.000	1.491	12.148	-10.657	0.000
		22	37.000	1.988	37.467	-35.479	0.000

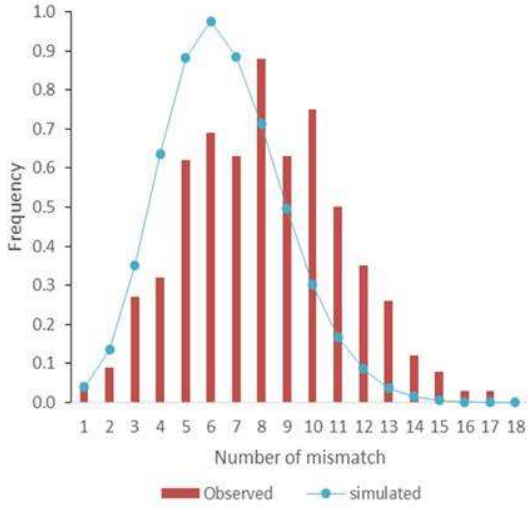

Figure 1: Chart showing mismatch distribution analysis of Red sokoto goat

Figure 1

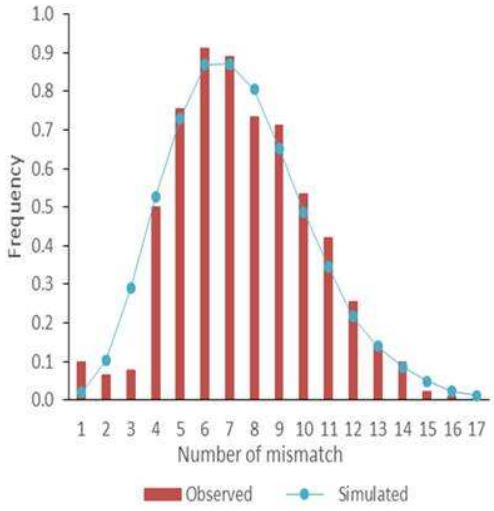

Figure 2: Chart showing mismatch distribution analysis of WAD goat

Figure 2

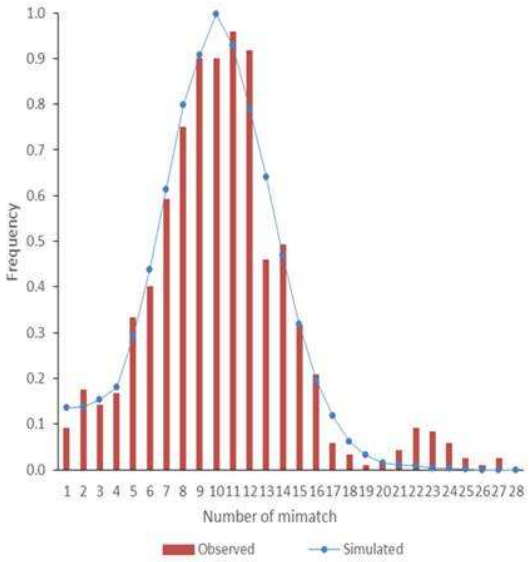

Figure 3: Chart showing mismatch distribution analysis of Sahel goat

Figure 3